Polymorphisms in swine candidate genes for meat quality detected by PCR-SSCP

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ABSTRACT - In order to identify SNPs in seven candidate genes for meat quality, PCR-SSCP experiments were carried out. Statistical analyses in a population of 240 individuals identified the animals constituting the extreme tails of the Gaussian distribution for three selected phenotypes: muscle compactness, fat thickness, and the principal component 1. Twenty-two of these animals were chosen for the molecular analyses. Several fragments in the candidate genes were tested by PCR-SSCP and many different patterns were found, indicating the presence of polymorphisms. The polymorphic fragments were sequenced and analyzed. *CRADD* was the most interesting gene, because many PCR-SSCP patterns were found in the samples. The results will be tested in another Italian Large White population to verify the segregation and the association of SNPs with phenotypes that influence meat quality.

Key words: SNP, Swine, Candidate genes, Meat quality.

Introduction - An important aim of pig selection in Italy is to obtain animals having a high aptitude for the PDO dry-cured ham production, such as Parma or S. Daniele ham.

Over the past years, advances in the porcine genetic map have led to valuable gene and trait information being discovered. Since that time, sequences for the pig genome have been generated from ESTs of cDNA clones from various tissues, the sequencing of candidate genes, and more recently large scale genomic sequencing projects. These efforts are also being directed to SNPs identification for future large scale association studies. In the next years, the efficiency and accuracy of the traditional pig selection schemes could be improved by the implementation of molecular data into breeding programs.

In a previous work, microarray experiments were carried out to find differences in gene expression levels between two pools of six individuals, constituting the extreme tails of the Gaussian distribution of seven adjusted phenotypes of 100 Landrace and Large White animals (Iacuaniello *et al.*, 2007). In this way, seven candidate genes that influence swine meat quality have been identified: inositol polyphosphate-1-phosphatase (*INPP1*), phosphoinositide-3-kinase regulatory subunit 2 (*PIK3R2*), protein tyrosine phosphatase (*PTPRD*), phospholipase c gamma 1 (*PLCG*1), casp2 and ripk1 domain containing (*CRADD*), cyclin-dependent kinase inhibitor 1c (*CDKN1C*), calpain small subunit 1 (*CAPNS1*).

The objective of this work was to identify informative SNPs in these seven candidate genes by means of PCR-SSCP (PCR-single-strand conformational polymorphism) analysis. PCR-SSCP is a rapid and sensitive method to detect polymorphisms in DNA (Chessa *et al.*, 2008; Kunhareang *et al.*, 2008). The products of PCR are heat-denatured to single stranded DNA (ssDNA), and the mutations or polymorphisms can be detected electrophoretically as mobility shifts resulting from a change in the conformation of ssDNA.

Material and methods - *Animals* - An Italian population of 240 pigs with a well-known genealogy was studied: pigs were full-sib or half-sib and were reared in the same conditions. After animals slaughtering, some phenotypes were evaluated: muscle compactness, marbling, colour uniformity, fat covering, colour, dorsal fat, thickness, ham fat thickness, vein system. Three phenotypes (muscle compactness, fat thickness, and the principal component 1) were considered for the selection of 22 individuals belonging to the extreme tails of Gaussian distributions to be used in the following molecular analyses. The principal component 1 was calculated considering the described phenotypes in order to summarize all the variables in new synthetic indicators of meat quality. Thus a "weighted index" called "meat quality index" was created in which the first component accounted for 35% of the variability. *RNA extraction and reverse transcription* - Total RNA was isolated from the skeletal muscle (*Longissimus dorsi*) of the 22 chosen animals using TRIzol® Reagent (Invitrogen) according to the manufacturer's instructions. RNA purity, integrity and concentrations were evaluated on the Agilent 2100 Bioanalyzer. cDNAs were obtained from RNA using The ImProm-II™ Reverse Transcription System kit (Promega). *PCR-SSCP analysis* - To amplify the cDNA of the seven candidate genes, many couples of primers were designed using Primer3 program (http://frodo.wi.mit.edu/) to obtain fragments with a length range of 200-300 bp. Prior to amplification, cDNA was denatured for 1 min at 95°C. Amplification consisted of 34 cycles at 94°C for 5 s, 56°C for 1 min, and 72°C for 1 min. After the cycles, an extension step (7 min at 72°C) was performed. The PCR products obtained were then heat-denatured and separated by polyacrylamide gel electrophoresis. Different conditions for detecting polymorphisms were used depending on the fragments amplified. The fragments showing different migration in the SSCP gels were sequenced and analyzed using Bioedit to identify the responsible SNPs.

Table 1. List of the animals chosen (ID) and the evaluations for the three selected phenotypes: compactness (Comp.), principal component 1 (PC 1), and ham fat thickness in mm (HFT). Compactness was evaluated assigning a score from 1 (very good) to 5 (insufficient).

Positive tail				Negative tail			
ID	Comp.	PC 1	HFT	ID	Comp.	PC 1	HFT
226	1	5.2510	42.5	275	3.5	-4.5433	12.5
129	1	3.5813	40	257	3.5		
91	1		42.5	164	3.5	-3.4026	
86	1		42.5	119	3.5		
81	1	3.5214	40	115	3.5		
79	1	3.4934	42.5	274		-3.1167	
44	1	3.1654		272		-4.5528	9
41	1	3.2666		225			15
				175			15
				249	4		
				224			15
				219		-3.2782	15
				170		-3.0721	15
				167	4		

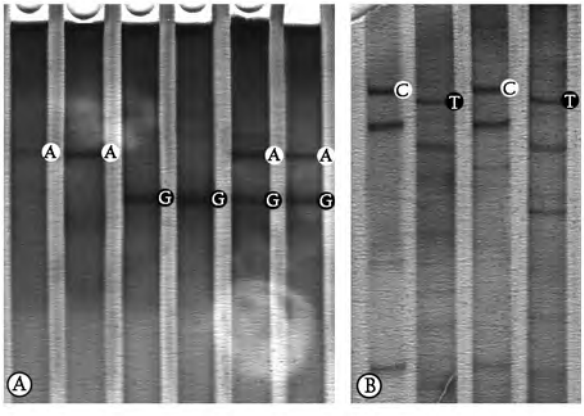
Results and conclusions - The seven candidate genes (*INPP1*, *PIK3R2*, *PTPRD*, *PLCG1*, *CRADD*, *CDKN1C*, *CAPNS1*) were tested in an Italian population of 240 pigs with a well-known genealogy, in order to identify SNPs associated with meat quality. Statistical analyses allowed to identify the animals constituting the extreme tails of the Gaussian distribution for the three selected phenotypes, muscle compactness, fat thickness, and the principal component 1 (PC 1). Twenty-two animals among these were chosen for SNPs searching: 8 animals belonged to the positive tail and 14 to the negative tail. Table 1 shows the list of the animals chosen and the respective evaluations.

To find differences in the seven selected genes between the 22 animals, PCR-SSCP experiments were performed. Many

different PCR-SSCP patterns were found as shown in Figure 1 and the most interesting fragments were sequenced and analysed in order to identify the SNPs responsible for the different SSCP patterns. At least 2 SNPs were found in the *CRADD* sequenced samples; the first one, an A/G transition, was found in the coding sequence, whereas the second one, a T/C transition, was found in the non-coding sequence. At least one T/C transition and a C/A transversion were found also in the coding sequence of *PTPRD*.

Our results will be tested in a different Italian Large White population, in order to verify the segregation and the association of SNPs with phenotypes that influence meat quality.

Figure 1. Examples of the PCR-SSCP results for *CRADD* (A) and *PTPRD* (B) genes. The polymorphic patterns for *CRADD* gene correspond to the A/G transition; the polymorphic patterns for *PTPRD* gene correspond to the C/T transition.



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